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Identification of novel mast cell genes by serial analysis of gene expression in cord blood-derived mast cells

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Abstract The gene expression profile of human cord blood-derived mast cells (MCs) was investigated using serial analysis of gene expression (SAGE). A total of 22 914 tags, representing 9181 unique transcripts, were sequenced. By selecting tags that were detected more frequently in MCs than in other tissues, genes characteristic of MCs were enriched. Reverse transcription-PCR and the high-density oligonucleotide array hybridization confirmed the validity of our SAGE result. About 70% of the selected genes were previously uncharacterized. Northern blot analysis showed the MC-specific expression of selected genes. This inventory will be useful to identify novel genes with important functions in MCs. **© 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Mast cell; Serial analysis of gene expression; Human; Transcript

1. Introduction

The functional features of a specific cell are characterized by a set of genes expressed within the cell. Most of previous studies have focused on a limited number of genes of interest. Although these approaches are useful, in order to fully understand the functions of a cell, it is necessary to know the comprehensive pattern of gene expressions.

Serial analysis of gene expression (SAGE) is a powerful technique to quantify thousands of transcripts simultaneously [1,2]. In this method, short sequence tags at a specific location within a transcript, which contain sufficient information to identify the gene, are generated, concatenated and sequenced. By cataloging tags along with their frequencies and identifying corresponding genes, we can estimate the expression level of thousands of genes.

Mast cells (MCs) have been understood as major effector cells in allergic diseases [3]. Recent findings suggest that MCs are also involved in non-allergic processes such as innate immunity by producing high levels of cytokines [4]. However little is known about other roles of MCs. We applied the SAGE technique to human MCs, because the overall understanding of gene expression pattern will lead to reveal novel functions in MCs.

SAGE technology has been utilized mainly to compare the different states of cells or tissues. Many SAGE data including various normal and neoplastic tissues are now publicly available [5,6]. One of the advantages of this method is that data from different sources are theoretically comparable because the amount of a transcript is expressed as the frequency of the tag. Here we demonstrate that MC-specific genes are effectively enriched from thousands of genes by comparing our SAGE data with those publicly available. Furthermore a large number of previously uncharacterized genes were identified from the inventory.

2. Materials and methods

2.1. Cell culture

Human umbilical cord blood (CB) samples were obtained from normal full-term deliveries with informed consents approved by the Hospital's Ethical Committee (National Children's Hospital, Tokyo, Japan). Human peripheral blood samples were also obtained from normal adult volunteers with written informed consents. Preparations of mononuclear cells and $CD34^+$ cells from these samples were described previously [7]. To obtain CB-derived MCs, the $CD34^+$ cells were cultured in Iscove's modified Dulbecco's medium (Gibco BRL, Grand Island, NY, USA) supplemented with stem cell factor (kindly provided from Kirin Brewery Co., Ltd., Maebashi, Japan) at 100 ng/ml and interleukin (IL)-6 (Kirin Brewery Co., Ltd.) at 50 ng/ml, 5×10^{-5} M 2-mercaptoethanol (Gibco BRL), 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco BRL) and 10% fetal calf serum (FCS) (Cansera, Rexdale, ON, Canada). After 8 weeks, the cells were further cultured in Iscove's modified Dulbecco's medium supplemented with stem cell factor at 200 ng/ml, IL-6 at 50 ng/ml and IL-4 (R&D Systems Inc., Minneapolis, MN, USA) at 10 ng/ml, 2-mercaptoethanol, antibiotics, and 10% FCS for 6 weeks. Maturation of MCs was confirmed by the immunocytochemical staining with anti-chymase and anti-tryptase antibodies as described previously [8]. Macrophages were obtained by culturing $CD34^+$ cells in the presence of GM-CSF (R&D Systems Inc.) at 10 ng/ml for 2 weeks. KCL-22, HEL, K562, KU-812-F and HMC-1 were maintained in our laboratory. Other cell lines were obtained from Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University.

2.2. SAGE

SAGE was performed according to the Serial Analysis of Gene Expression Detailed Protocol version 1.0c and analyzed using SAGE analysis software 3.04b generously provided by K.W. Kinzler (Johns Hopkins University, Baltimore, MD, USA). Minor modifica-

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Abbreviations: SAGE, serial analysis of gene expression; MC(s), mast cell(s); CB, cord blood; Btk, Bruton's tyrosine kinase; HDC, histidine decarboxylase; EST, expressed sequence tag; PBMNC(s), peripheral blood mononuclear cell(s); PG, prostaglandin; MITF, *mi* locus-encoded transcription factor; LT, leukotriene

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tions were made as follows. Poly(A)$^+$ RNA ($\sim$1 μg estimated by dot quantification method) purified from 75 μg of total RNA was used for SAGE. PCR amplification of ditags was performed for 18 cycles first, the band containing ditags were isolated, and re-amplified for 10 cycles. For the identification of corresponding genes, tag sequences were matched with the SAGE tag database extracted from GenBank primate sequence files (gbpri1, 2, 3, 4 and 5; release 117.0) using the SAGE database utility. Unigene data for tag to gene mapping were downloaded via NCBI FTP site (ftp://ncbi.nlm.nih.gov/pub/sage/map/Hs/Nla3/) and also utilized for the gene identification. SAGE libraries used for comparison with that of MC were NC1, NC2, BB542, NHA (fifth), normal pool (sixth), normal cerebellum, Duke thalamus, mammary epithelium, Br N, HOSE4, IOSE29-11, 293-CTRL, Chen Normal Pr, Duke HMVEC from NCBI SAGE Library Browser (www.ncbi.nlm.nih.gov/SAGE/sagelb.cgi) and skeletal muscle [9] from Rochester Muscle Database web site (www.urmc.rochester.edu/smd/CRC/Swindex.html).

2.3. Reverse transcription (RT)-PCR and RNA blot hybridization analysis

Total cellular RNAs from CB-derived MCs, CB-derived macrophages and various human cell lines were isolated using ISOGEN (Nippon Gene, Tokyo, Japan). Total RNAs from human tissues were purchased from OriGene Technologies, Inc., Rockville, MD, USA. For RT-PCR, 1 μg of total RNA was primed with oligo(dT)$_{12-18}$ (Gibco BRL) and reverse-transcribed using Superscript-II (Gibco BRL) according to the manufacturer's instruction. The following pairs of primer were used: CDw52, 5′-AAG CGC TTC CTC TTC CTC CTA CTC-3′ and 5′-GTC TGG CAT CAA CCC CTC CCA AAG-3′; HSPC022, 5′-GCC CCA GGA CCC ATT AGG-3′ and 5′-CTC TCA CAG GTA AGT GCA GC-3′; AW182663, 5′-GTT TCT TGG ATA ATG GGC AG-3′ and 5′-CAA AGT CCT AGT TAT AGA TAT C-3′; Bruton's tyrosine kinase (Btk), 5′-TTG AAC GTG GGA GAA GAG GCA G-3′ and 5′-CTC AGG CGG TAG TGG CTT TTT C-3′. The sequences of primers for histidine decarboxylase (HDC) and β-actin were described previously [10]. Cycling conditions are as follows: denaturation for 30 s at 94°C, annealing for 30 s at 50°C (AW182663), 55°C (HSPC022) or 60°C (CDw52, HDC, Btk and β-actin) and elongation for 1 min at 72°C for 30 cycles (CDw52, HDC, Btk, β-actin and AW182663) or 35 cycles (HSPC022). For Northern blotting, 10 μg of total RNA was separated through 1% agarose gel under denaturing conditions and blotted to Hybond N$^+$ (Amersham Pharmacia Biotech, Tokyo, Japan). The RT-PCR products or the inserts isolated from expressed sequence tag (EST) clones (Genome Systems Inc., St. Louis, MO, USA) were labeled with [α-^{32}P]dCTP using labeling kit (rediprime, Amersham Pharmacia Biotech). Hybridization and washing were performed as described previously [10].

2.4. High-density oligonucleotide array hybridization

The mixtures of three different batches of CB-derived MCs and of six different batches of peripheral blood mononuclear cells (PBMNCs) were used. Total RNAs obtained from CB-derived MCs and PBMNCs were treated for producing cRNA. The produced cRNA was hybridized with GeneChip® Human Genome U95A Array (Affymetrix, Santa Clara, CA, USA) consisting of $\sim$12 000 high-density oligonucleotide probe arrays. The gene expression was analyzed by using the computer software, GeneChip® Analysis Suite (Affymetrix).

3. Results and discussion

3.1. Summary of SAGE in CB-derived human MCs

SAGE was performed on poly(A)$^+$ RNA purified from human CB-derived MCs that were comprised from 100% tryptase and 100% chymase positive cells determined by immunocytochemical staining. A total of 22 914 tags were collected from CB-derived MCs. After excluding linker-derived sequences, 21 937 tags remained. These remaining tags corresponded 9181 different transcripts, with overlapping 2539 tags. Tags counted more than 50 times ($>$0.23% of total transcripts) are shown in Table 1. Most of them were derived from genes that are expressed ubiquitously throughout various tissues, such as those related to energy metabolisms (ATPase 6/8, cytochrome *b*, cytochrome *c* oxidase, NADH dehydrogenases), protein synthesis (elongation factor, ribosomal proteins) or cytoskeletal components (β- and γ-actin, vimentin). Among them only one tag matched a gene characteristic of MC, MC carboxypeptidase A (0.24%).

Table 1
Genes abundantly expressed in CB-derived human MCs[a]

Tag sequence	Count	Gene (GenBank accession no.)
GAAATCAAAA	497	Multiple matches[b]
CACCTAATTG	254	*ATPase 6/8* (*X93334*)[c]
TGTGTTGAGA	228	Elongation factor 1 α (X03558)
CACTACTCAC	219	*Cytochrome b* (*X93334*)[c]
CCCATCGTCC	197	*Cytochrome c oxidase subunit II* (*X15759*)[c]
ACTTTTTCAA	170	DKFZp434D1735 (AL133037)
TTCATACACC	167	FLJ20617 fis, clone KAT05223 (AK000624)
CTAAGACTTC	163	*16S rRNA* (*X93334*)[c]
AGCCCTACAA	148	*NADH dehydrogenase 3* (*X93334*)[c]
TGATTTCACT	146	Autonomously replicating sequence (L08441)
ACTAACACCC	136	PACAP type-3/VIP type-2 receptor (U18810)
ACCCTTGGCC	133	*NADH dehydrogenase 1* (*X93334*)[c]
CCTGTAATCC	122	Multiple matches[b]
GTGAAACCCC	111	Multiple matches[b]
CCCTGGGTTC	102	Ferritin L chain (M11147)
CCTCAGGATA	100	*NADH dehydrogenase 5* (*X93334*)[c]
AAAAAAAAAA	86	Multiple matches[b]
GCTTTATTTG	86	β-Actin (X00351)
TTGGTCCTCT	75	Ribosomal protein L41 (AF026844)
AAGACAGTGG	68	Ribosomal protein L37a (L22154)
TCCAAATCGA	62	Vimentin (X56134)
TAGGTTGTCT	57	Translationally controlled tumor protein (X16064)
CTAGCCTCAC	56	Cytoskeletal γ-actin (X04098)
CCACTGCACT	54	Multiple matches[b]
GCGGTTGTGG	54	Lysosomal-associated multitransmembrane protein LAPTm5 (U51240)
ATGTGTAACG	52	CAPL protein (M80563)
GAAATACAGT	52	Cathepsin D (M11233)
GCCGAGGAAG	52	Ribosomal protein S12 (X53505)
TGGCTTCATC	52	MC carboxypeptidase A (M27717)
CACAAACGGT	51	Ribosomal protein S27 (U57847)
AAGGACCTTT	50	ESTs (AA458972)
TTGGGGTTTC	50	Ferritin heavy chain (L20941)

[a]Tags detected at least 50 times are listed in descending order.
[b]Multiple matches means the tag matched at least two genes and they could not be distinguishable.
[c]Italic denotes the mitochondrial gene.

3.2. Enrichment of MC-specific genes by comparison with SAGE libraries from other tissues

The SAGE data of MCs were compared with 15 other SAGE libraries from various tissues or cells including brain, colon, mammary gland, ovary, kidney, prostate and skeletal muscle. The frequencies of each tag (tag counts per total tag counts) in all libraries were calculated and that in MC was compared to those in other tissues. Tags, which were detected frequently in MCs by more than 5-fold compared to those in other tissues, were chosen. After excluding tags appeared only once, 246 different transcripts remained. Those that were detected at least six times ($>$0.03%) are shown in Table 2. The genes with known function in MCs are proteases (carboxypeptidase A, cathepsin G and tryptase), enzymes relating to

Table 2
Tags preferentially detected in MC compared to other tissues[a]

Tag sequence	Count	Gene (GenBank accession no.)
GAAATCAAAA	497	Multiple matches[b]
GCGGTTGTGG	54	Lysosomal-associated multitransmembrane protein LAPTm5 (U51240)
TGGCTTCATC	52	Carboxypeptidase A (M27717)
TGAGATTGAT	40	NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (L76465)[c]
AGGAGGGGAA	38	Cathepsin G (M16117)
AGTCCTTGAA	38	c-kit (X06182)
CACTTGGTGA	37	ESTs (AA913279)[d]
CTGGCGCGAG	36	Rho GDP-dissociation inhibitor 2 (X69549)
GACAGTTGTT	28	GATA2 (M68891)
CGACGAGGAG	26	Epithelial membrane protein-3 (X94771)
GGGGCAACAG	24	**CDw52 antigen (X62466)**[e]
CCAGGGCGAC	22	Tryptase-II/III (M33492/3)
TGGAAGCATC	22	**HSPC022 (AF077208)**[e]
AGGATGACCC	17	HSPC113 (AF161462)
GGAAATCAAA	17	No match
GGCTTCCTGG	17	EST (AW193344)
GACTGGAAAA	16	ESTs (AA995596)[d]
ATGCCGCCAG	13	**ESTs (AW291847)**[d,e]
ATTTCTTTTA	13	**Id-2 (M97796)**[e]
CTTCTGGGGA	13	**RhoG (X61587)**[e]
ATATCTATAA	12	**ESTs (AW182663)**[d,e]
GTTAAATGAC	11	ESTs (AA291716)[d]
AAACACCTTA	9	Breast cancer suppressor candidate 1 (AF002672)
AGTAGTGGGC	9	ESTs (AW338862)[d]
GGCTCACTTT	9	DUSP6 (AB013382)
TACAAATAGG	9	Clone 25194 (AF131784)
AACTCGGTGG	8	**ESTs (AI589086)**[d,e]
AATCCTTACT	8	P2x1 receptor (U45448)
CTCCCTCCCC	8	Glutathione-requiring PGD synthase (D82073)[c]
GTACATAAGA	8	**ESTs (AA741162)**[d,e]
TTAATCTGAG	8	KIAA0006 (D25304)
AAGCTAATAA	7	PG endoperoxide synthase (M59979)[c]
AATGCTTTTA	7	CYP1B1 (U03688)
ACATTCTTTT	7	NMB (X76534)
ATATATAACA	7	HDC (X54297)
CATTTGCACT	7	CD69 (L07555)
GCCTTTCTAT	7	EST (W95106)
GTTTCAAACG	7	ESTs (AI797884)[d]
AAGACCACTG	6	EST (AA953113)
GATGAAGAAT	6	Btk (X58957)
GCATAGTTGG	6	15-Hydroxyprostaglandin dehydrogenase (X82460)[c]
GCGTCCTGCC	6	Linker for activation of T cells (AF036905)
GCTGAGTGCA	6	Breast cancer nuclear receptor binding auxiliary protein BRX (AF126008)
TCTGTTTCCT	6	ESTs (AW014827)[d]

[a]Tags, which were expressed in MC at least 5-fold greater than any other tissues, were selected and those counted at least six times are listed in order of descending abundance.
[b]The tag matched several genes.
[c]PGD_2-related genes.
[d]If the tag matched two or more ESTs, the GenBank accession number for one of them is shown.
[e]Genes in boldface were confirmed to be expressed in CB-derived MCs by Northern blot analysis.

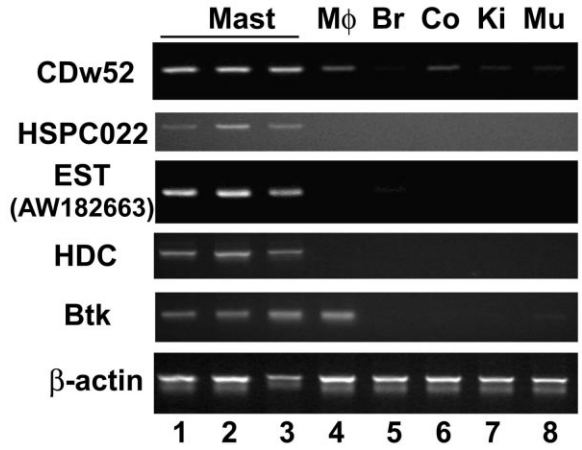

Fig. 1. RT-PCR analysis. First strand cDNAs were synthesized from CB-derived MCs of three different donors (lanes 1–3, Mast), CB-derived macrophages (lane 4, Mϕ) and four different human tissues (lanes 5–8; Br, brain; Co, colon; Ki, kidney; Mu, muscle) and amplified with the gene-specific primers. The experimental conditions were described in Section 2.

mediator synthesis or degradation (prostaglandin (PG) endoperoxide synthase, PGD synthase, HDC and 15-hydroxyprostaglandin dehydrogenase), signal transduction proteins (c-kit, rhoG, rho GDP-dissociation inhibitor 2, Btk and linker for activation of T cells) and a transcription factor (GATA2).

To check the validity of our SAGE data, the expressions of representative genes were examined by RT-PCR and oligonucleotide microarray. First, the expressions of six genes in CB-derived MCs from three different donors were analyzed by RT-PCR. As controls, CB-derived macrophages and RNAs from various human tissues were also examined (Fig. 1). All the genes except β-actin were expressed higher in all three CB-derived MCs (lanes 1–3) than in brain, colon, kidney and muscle, in good agreement with the comparison of SAGE data. Two genes (CDw52 and Btk) were expressed in macrophages (lane 4) as well as MCs. Next, the gene expression profiles were compared between CB-derived MCs and PBMNCs using oligonucleotide microarray. In this method, the amount of a transcript is expressed as the average difference between the fluorescent intensities of matched microprobes and those of single-nucleotide mismatched microprobes. The expression levels of 20 representative genes of interest and β-actin are shown in Table 3. Many genes in Table 2 were expressed more abundantly in CB-derived MCs than in PBMNCs (Table 3), suggesting that our selection effectively enriches MC-specific genes. The most abundant tag in the MC SAGE library, which appeared 497 times, simultaneously matched several genes including α-, β-tryptases and sialic acid binding Ig-like lectin-5, which were indistinguishable by the tag sequence (Tables 1 and 2). Because in oligonucleotide microarray analysis the average difference of tryptase gene in CB-derived MCs was much higher than that of other genes (Table 3), we assume most of these tags are derived from α- or β-tryptases.

Because genes in Table 2 were selected by comparing the SAGE data of MCs against those of non-hematopoietic tissues, it may contain genes generally expressed among hematopoietic cells. In fact, lysosomal-associated multitransmembrane protein LAPTm5 or CDw52 in Table 2 are also found

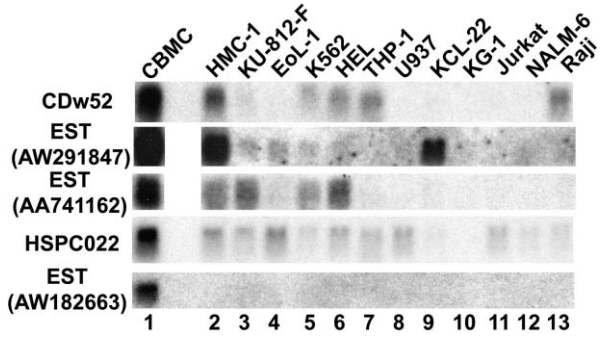

Fig. 2. RNA blot hybridization analysis. Each lane contains 10 μg of total RNA from CB-derived MCs (CBMC, lane 1), HMC-1 (lane 2), KU-812-F (lane 3), EoL-1 (lane 4), K562 (lane 5), HEL (lane 6), THP-1 (lane 7), U937 (lane 8), KCL-22 (lane 9), KG-1 (lane 10), Jurkat (lane 11), NALM-6 (lane 12) and Raji (lane 13). The equality of the amount of applied RNA was confirmed by staining the gel with acridine orange. Two blots were used: one for CDw52, HSPC022 and EST (AW182663) and the other for EST (AW291847) and EST (AA741162). After probing with one, blots were stripped and reprobed with another.

in SAGE libraries derived from GM-CSF- or M-CSF-induced macrophages [11]. In addition, LAPTm5 and rhoG were expressed substantially in PBMNCs as well as CB-derived MCs (Table 3). Therefore the inventory shown in Table 2 contains MC-specific genes and some genes generally expressed among hematopoietic cells.

3.3. Identification of novel MC-specific genes

To check the expression profile of the several genes from Table 2 among hematopoietic lineages, we performed RNA blot hybridization analysis using various hematopoietic cell lines. Most of the genes examined showed higher expression in CB-derived MCs (Fig. 2, lane 1) than in other cell lines (lanes 2–13). The expressions of CDw52, EST (AW291847) or EST (AA741162) were relatively restricted to several cell lines, while HSPC022 expressed ubiquitously among hematopoietic cell lines. The expression of EST (AW182663) was very specific to CB-derived MCs. These results suggest that the listed genes chosen by this criteria were quite specific to MCs and they are useful as a catalog for the MC-specific genes.

One of the most important results of this exploration is finding a large number of unnamed genes such as EST, HSPC or KIAA genes. Among 246 selected tags, about 30% corresponded to previously characterized genes. About a half of remaining tags matched unknown genes including ESTs and the other half did not have any match. We have demonstrated the enrichment of genes characteristic of MCs by selecting tags preferentially detected in MC SAGE library compared to those of other tissues (Table 2). In addition, the analysis of predicted amino acid sequences for HSPC or KIAA genes found in the selected genes against PROSITE database revealed putative functional domains including Src homology 3, Calponin homology, pleckstrin homology, Rho-GAP and Dbl domains. This enrichment of genes characteristic of MCs suggests that some of the uncharacterized genes in this list may have novel important functions in MCs.

Genes with known functions in other cell types are also found in this SAGE library. The helix-loop-helix protein, Id2, is one of those genes. Proteins of this family are known to function as a transcriptional regulator by dimerization with other basic helix-loop-helix DNA binding proteins [12]. It is reported that only Id2 was expressed in MCs among four members of Id protein family [13]. Tags corresponding to LYL-1 and SCL, both of which are basic helix-loop-helix

Table 3
Comparison of gene expressions between CB-derived MCs and PBMNCs using oligonucleotide microarray[a]

Gene (GenBank accession no.)	Average difference[b]		
	CB-derived MCs	PBMNCs	Ratio[c]
Tryptase (M30038)	21 444	1	22 772.4
c-kit (X06182)	3 883	1	4 123.5
Cathepsin G (M16117)	7 072	7	1 072.9
NMB (X76534)	9 007	31	308.5
Breast cancer suppressor candidate 1 (AF002672)	6 059	31	207.6
NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (L76465)	6 259	79	84.1
15-Hydroxyprostaglandin dehydrogenase (X82460)	4 423	136	34.5
Carboxypeptidase A (M73720)	6 956	297	24.9
GATA2 (M68891)	7 223	464	16.5
P2x1 receptor (U45448)	2 018	210	10.2
HDC (D16583)	3 579	424	9.0
PG endoperoxide synthase (M59979)	1 027	145	7.5
CYP1B1 (U03688)	3 279	881	4.0
Chymase (M69136)	349	110	3.4
Id-2H (D13891)	8 636	3 151	2.9
KIAA0006 gene (D25304)	3 370	1 319	2.7
Lysosomal-associated multitransmembrane protein LAPTm5 (U51240)	12 940	10 574	1.3
RhoG (X61587)	1 859	2 161	0.9
TNF (M16441)	65	199	0.3
IL-4 (M13982)	23	80	0.3
β-Actin (X00351)	18 063	19 182	1.0

[a]Genes are listed in the order of descending ratio.
[b]Average differences were obtained by subtracting the fluorescent intensities of mismatched microprobes from those of matched microprobes. If the average difference was 0 or less, it was set to 1.
[c]Ratio of the average difference in CB-derived MCs to that in PBMNCs normalized by the average difference of β-actin.

proteins known to play important roles in hematopoietic cells, were also detected (LYL-1, four; and SCL, one) in MC SAGE library, suggesting possible roles of these helix-loop-helix proteins in MCs. Among the transcription factors, *mi* locus-encoded transcription factor (MITF) and GATA2 are known to be particularly important in mouse MC gene expressions. While MITF is important in the early stage of MC development [14], GATA2 is involved in the transcriptions of various genes in mature MCs [15]. Because we made the SAGE library from mature MCs, it is in good agreement with these previous reports that the tag corresponding to GATA2 was detected much more frequently than MITF (GATA2, 28 and MITF, two). The abundance of GATA2 tag was highest among known transcription factors in MC SAGE library (Table 2). Oligonucleotide microarray analysis also showed high GATA2 expression in CB-derived MCs (Table 3). These results may reflect the significant roles of GATA2 in mature MCs.

MCs release newly generated mediators such as PGD_2, leukotriene (LT) B_4 or LTC_4 upon activation as well as preformed mediators [3]. We found the tags for enzymes relating to synthesis and degradation of PGD_2 appeared frequently (Table 2), while those involved in synthesizing lipoxygenase products such as LTB_4 or LTC_4 were detected rarely (5-lipoxygenase, zero; LTC_4 synthase, one; and LTA_4 hydrolase, one). This suggests a possibility that PGD_2 is constitutively synthesized and may have some functions in unstimulated MCs. Tags for some preformed mediators or cytokines expressed in MCs in protein level [3] were scarcely detected (TNF-α, zero; IL-4, zero; and chymase, one). The oligonucleotide microarray analysis also showed the average differences of these genes were lower than those of other genes (Table 3). These inconsistencies between mRNA and protein levels suggest that all the gene products are not regulated in transcriptional level as the study in which a comprehensive and quantitative comparison of mRNA and protein expression levels in yeast showed that the protein level does not always correlate with its mRNA level [16].

In conclusion, we have explored the comprehensive gene expression profile in human MCs. This is the first study that provides the information of the species and the amount of genes expressed in human MCs. It also identified a lot of uncharacterized genes. The functional analyses of these genes would reveal various novel roles of MCs.

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